

Perspective

Genetics of the Musculoskeletal System: A Pleiotropic Approach

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ABSTRACT: The risk of osteoporotic fracture can be viewed as a function of loading conditions and the ability of the bone to withstand the load. Skeletal loads are dominated by muscle action. Recently, it has become clear that bone and muscle share genetic determinants. Involution of the musculoskeletal system manifests as bone loss (osteoporosis) and muscle wasting (sarcopenia). Therefore, the consideration of pleiotropy is an important aspect in the study of the genetics of osteoporosis and sarcopenia. This Perspective will provide the evidence for a shared genetic influence on bone and muscle. We will start with an overview of accumulating evidence that physical exercise produces effects on the adult skeleton, seeking to unravel some of the contradictory findings published thus far. We will provide indications that there are pleiotropic relationships between bone structure/mass and muscle mass/function. Finally, we will offer some insights and practical recommendations as to the value of studying shared genetic factors and will explore possible directions for future research. We consider several related questions that together comprise the general paradigm of bone responses to mechanical loading and the relationship between muscle strength and bone parameters, including the genetic factors that modulate these responses. We believe that further progress in understanding the common genetic etiology of osteoporosis and sarcopenia will provide valuable insight into important biological underpinnings for both conditions and may translate into new approaches to reduce the burdens of both conditions through improved diagnosis, prevention, and early targeted treatment.

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INTRODUCTION

THE RISK OF osteoporotic fracture can be viewed as a function of the ability of human bone to withstand loads. Skeletal loading conditions are largely dominated by muscle action, more so at younger ages. With advanced age, bones undergo a profound decline in their responsiveness to mechanical loads from exercise, which probably corresponds to the aging-related changes in cellular and hormonal mechanisms and the decline of muscle input. Because bone and muscle cells share the same mesenchymal precursor, it is thus reasonable to hypothesize that adult bone strength and muscle mass share genetic determinants.

The aim of this perspective paper is therefore to provide evidence from experimental and epidemiological studies for a new conceptual framework for the biological, especially genetic, mechanisms underlying an interaction between bones and muscles in adults. We will start with an overview of accumulating evidence that muscle mass and physical exercise produce effects on the skeleton. We will then provide indications that the genetic influences are shared between the muscles and bone strength. Finally, we will offer some insights on the possible nature of shared

genetic factors and conclude with some potential directions and practical recommendations for future research in this field. We thus consider several related questions that together comprise the general paradigm of bone responses to mechanical loading and the relationship between muscle strength and bone parameters. (1) What is the evidence that the age-related decline in bone and muscle mass have a common etiology? Is it an artifact of general aging? (2) How do aging bones respond to the changes in muscle input/strength? (3) What genetic factors modulate these responses? (4) What is the best way to study the pleiotropic relationships between bone structure/mass and muscle mass/function?

In this perspective, we attempt to answer these important conceptual questions. Further progress in understanding common genetic etiologies of osteoporosis and sarcopenia may ultimately lead to approaches to reduce the burdens of both conditions through improved diagnosis and early-targeted treatment.

AGING OF BONE AND OSTEOPOROSIS

The process of involution of the musculoskeletal system seems to be a general phenomenon and a “normal” manifestation of tissue atrophy with age.⁽¹⁾ Bones undergo a

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profound decline with age in their responsiveness to mechanical loads from exercise,⁽²⁾ a decline that also probably corresponds to the decrease in muscle input that arises from the shortage in energy and fiber loss.

After the skeleton has reached maturity, bone remodeling is responsible for the complete replacement of old bone tissue with new tissue.⁽³⁾ With advanced age, the placement and quality of new bone are altered. This alteration is caused by the change in areas of remodeling due to disease, accumulated microtrauma, and by variations in muscle strength and direction of applied force, caused by various kinds of work and leisure activity. Catabolic illness, such as surgery, injury, palsy,⁽⁴⁾ and immobilization (bed rest or space flight⁽⁵⁾), may contribute to rapid bone degeneration. The net result of the age-related bone changes in older persons is chronic degenerative disease, which is characterized by the loss of bone mass and bone strength, development of fragility, and the alteration of bone morphology.

The most widely used and reliable clinical predictor of an osteoporotic fracture remains areal BMD,⁽⁶⁾ as evaluated by DXA. The strength of bones is determined not only by the amount of bone but also by the spatial distribution of bone tissue. A growing body of evidence indicates that bone geometry contributes substantially to bone strength and fracture risk.^(7,8) Femoral geometry may be assessed noninvasively also by DXA-based hip structural analysis (HSA). The more sophisticated technique of QCT provides valid measures of volumetric BMD (especially trabecular) and precise estimates of the bone structural traits; however, it is not completely clear whether it is superior to DXA in predicting fracture risk.⁽⁹⁾

AGING OF MUSCLE AND SARCOPENIA

Similar to bone, muscle tissue deteriorates with the age. Age-associated loss of muscle fibers, fatty degeneration, and decreased number of functioning motor units cause decline in muscle quality (i.e., force generated per unit of muscle mass).⁽¹⁰⁾ The weight of skeletal muscle comprises ~45% of body weight at age 21–30 yr and only ~27% at age 70+ yr.⁽¹¹⁾ Sarcopenia (muscle wasting) is manifested by decreases in muscle strength and muscle mass with age, mainly as a result of the alterations in muscle morphology. This is caused mostly by a decreased proportion and cross-sectional area of type II (fast-twitch) fibers; however, single fiber analysis suggests that the contractile proteins also become less effective with age.⁽¹²⁾ Hand grip strength is known to be associated with muscular functioning in other muscle groups and with activities of daily living. Reduction in muscle mass and hand grip strength is ~25–30% between ages 30 and 70 yr.⁽¹¹⁾ Hand grip strength predicts disability^(13,14) later in life and may also be a good predictor of future deficits in the lower extremities leading to locomotor and balance problems.

Although muscle biopsy is best able to quantify the magnitude of sarcopenia, obtaining such samples is not feasible for research in large human populations. Noninvasive imaging modalities such as QCT and MRI are particularly useful, providing quantitative estimates of muscle mass.⁽¹⁵⁾ Muscle density measured by QCT is a reliable and valid

measure of muscle degeneration that accompanies sarcopenia.^(16,17) The area and radiographic density of the trunk musculature have been associated with both lower back pain and level of physical function in elderly individuals.^(18,19) DXA-measured lean mass also serves as an indirect indicator of mechanical loading on bone.⁽²⁰⁾ Higher lean mass is associated with greater muscle strength and better functioning^(21–23); leg lean muscle mass measured by DXA has been shown to be associated with mobility disability.^(21,24,25)

RELATIONSHIP BETWEEN OSTEOPOROSIS AND SARCOPENIA

There are many indications that sarcopenia coexists with osteopenia.⁽²⁶⁾ The key to understanding the etiology of osteoporosis and sarcopenia lies in the recognition that bones and muscles act as two parts of the same functional unit of the musculoskeletal system, as proposed by Frost and colleagues^(27,28); therefore, loading effects on muscle will also influence bone. Sievänen⁽²⁹⁾ postulated that “It is quite clear that the behavior of such a complex system is qualitatively and quantitatively different from what can be inferred from observations and phenomena in isolated units of the whole system.” There are both theoretical (evolutionary and allometric) and empirical (basic biologic and clinical epidemiological) underpinnings that support this view and provide evidence that the musculoskeletal system develops, functions, and ages as a whole.

EVOLUTIONARY VIEW: ALLOMETRY AND PLEIOTROPY

There are three major ontogenetic periods in the bone and muscle relationship: patterning in embryonic life, allometric growth in the postnatal period, and the homeostatic relationship in adult life.⁽³⁰⁾ For recent and comprehensive reviews of embryonic sources of muscle and bone development as well as progenitor cells, see Refs. 2 and 31. In brief, muscle and bone cells share a common mesenchymal precursor. Furthermore, their production for the needs of organogenesis is regulated by a concerted action of genes, which is clearly evident during intrauterine development. The above concept of a universal biomechanical unit^(27,28) was recently used by Matsuoka et al.,⁽³¹⁾ who proposed to identify genes involved in the cellular modularity of the muscle and bone.

The theory of allometry fully supports our assertion that genetic influences on bone and muscle are exerted in a pleiotropic manner. Allometry is usually defined as the form/size/shape covariance; the relationship between the growth and size of one body part to the growth and size of the whole organism. In regard to the musculoskeletal system, we expect that morphological traits, such as joint shapes and sizes, long bone diaphyseal morphology, muscle–bone interfaces (entheses), and bone–muscle lever orientations, would tend to covary significantly with gross morphological parameters because of the need to maintain an integrated, functioning system in light of variation in overall form and shape.⁽³²⁾ In theory, the pattern

of (co)variation is expected to evolve to match fitness demands,⁽³³⁾ and alleles whose pleiotropic effects contribute to the attainment of appropriate proportions by interdependent parts that will generally be favored by natural selection.⁽³⁴⁾

Models of allometric scaling of bone variables have been proposed.^(35,36) In particular, Nevill et al.⁽³⁷⁾ supported the view that the BMC of the L₂–L₄ vertebrae develops proportionally to lean body mass in healthy adolescents. This complex system needs to be regulated by a concerted action of genes; also, it is very improbable that bone tissue homeostasis would be maintained by means of a single, simple mechanism.⁽²⁾ There is a need for genetic mechanisms to be redundant, pleiotropic, and polygenic for safety reasons.^(29,33)

The spectrum of pleiotropic effects of a gene on a morphological trait may include both “direct” and “indirect” effects (through some intermediate mechanism), with a possible continuum in between.⁽³⁸⁾ It may be a single gene that can trigger changes in bone anatomy, and as a result, affect muscle architecture. For example, the genetically affected rate and/or duration of mitosis in the proliferative zones of femoral growth plates⁽³⁹⁾ or delayed mineralization of growth plate cartilage may lead to elongation of the femoral neck (with reduction of the neck shaft angle and height of the greater trochanter); both changes involve functional adaptations of soft tissues to maintain fitness in walking.⁽³⁴⁾ However, it is more plausible that slight changes of systemic control factors during development (e.g., small modulations of the growth hormone [GH] axis can generate fully coordinated morphological change).⁽³⁴⁾ For example, there is a plethora of indications that GH and related factors (such as insulin-like growth factor 1 [IGF1]) may exert both direct anabolic effects on bones, as well as indirect effects, through influences on muscle (see *Candidate genetic mechanisms for bone geometry and muscle mass*).

Allometric shifts do not occur rapidly in an evolutionary and ontological sense; this is a long and involved process. Although bone has often been considered an optimal structure under given loading conditions, it cannot immediately attain an optimum under a varying load, because of the slow speed of adaptation.⁽⁴⁰⁾ Recent empiric and theoretical work suggests that there is no single optimal bone architecture; instead, many different architectural solutions produce adequate bone strength.^(41,42) An interplay of genetic predisposition, environmental demands, and stochastic processes is thus expected to shape bones in vivo.

The mineral mass and lean mass content of the human body may also be functionally associated.⁽⁴³⁾ This has led to studies of functional (biomechanical) as well as genetically determined allometry.^(32,44) If mineral mass and lean mass were functionally driven, morphologic traits would be related due to environmental stimuli. Indeed, and not surprisingly, body mass emerges as the strongest single predictor of femoral cross-sectional geometry, explaining 82% of the variance in femoral second moments of area.⁽⁴⁵⁾ It is also possible that there are specific, local (i.e., nonallometric) increases in the size of the bones and joints as an isolated

adaptation to load-bearing. Such adaptations that have no antecedent differences in pattern formation and occur due to environmental stimuli, are probably epigenetic.⁽³⁴⁾ Bone at different locations in the skeleton maintains very dissimilar strain thresholds for initiating modeling.⁽⁴¹⁾ The most widely exploited examples are the difference in size, shape, and moment of inertia between the dominant and non-dominant arms in tennis players^(46,47) and the asymmetry in metacarpal measurements due to hand dominance.⁽⁴⁸⁾ Even if there is an obvious environmental trigger, there should be a molecular substrate in bone responsible for recording and responding to the action of muscles; this susceptibility to outside triggers should be genetically determined.

EMPIRIC EVIDENCE (OBSERVATIONAL AND EXPERIMENTAL)

Inter-relationship between muscle mass/strength and bone mass/geometry in adults

Studies of both humans (adults and children) and laboratory animals have documented a strong, positive correlation between muscle strength and bone mass.⁽⁴⁹⁾ According to in vitro studies, the ligamentous lumbar spine ex vivo, without assistance from muscles, buckles under compressive loads <90 N, whereas the in vivo spine can withstand loads >6000 N during daily tasks⁽⁵⁰⁾ and up to 18,000 N in competitive power lifters.⁽⁵¹⁾ The ability of the in vivo spine to tolerate such high loads is mainly attributable to the dynamic stabilizing capacity of the trunk musculature.⁽¹⁹⁾ Indeed, there are numerous slips of muscle, which either connect to individual lumbar vertebrae, ribs, or attach inferiorly to the pelvis and/or sacrum, or to the femora (psoas major).⁽⁵²⁾ Because so many morphological features interact to create the mechanical environment of the lumbar dorsal vertebra or proximal femur, analysis of its structure requires an integrated approach.⁽³⁴⁾

Skeletal loads are dominated by muscle action, and the mechanostat theory postulates that bone strength adapts primarily to muscle forces.⁽²⁸⁾ In brief, the mechanostat theory proposes that loads that generate strains above some hypothetical threshold stimulate growth and inhibit haversian remodeling, whereas loads that generate strains below another threshold inhibit growth and stimulate loss of bone, thus helping bone to redistribute in space. Indeed, it was postulated that bone cross-sectional properties mostly reflect shape adaptations to only the most vigorous forms of loading rather than to typical habitual activities.⁽²⁾ However, there is evidence that the threshold of response varies with location in the bone (e.g., diaphysis versus epiphysis⁽⁵³⁾). Moreover, it has been hypothesized that extremely small strains, if induced at a sufficiently high frequency, are strong determinants of bone morphology.⁽⁵⁴⁾ In essence, this hypothesis has evolved from the demonstration that the spectrum of strain within functionally loaded bones can be characterized as having an inverse power-law (1/f) relationship between the magnitude of strain events and the incidence of these events.⁽⁵⁵⁾ As such, the strain history of a bone is characterized by a very few large strain events (e.g., four events per 24 h that exceed 2000 microstrain⁽⁵⁶⁾), slop-

ing linearly to 100,000s of very small strain events, each well below 10 microstrain, which arise through the dynamics of muscle contraction. This suggests that the bone tissue depends as much on the persistent, low magnitude strains that arise through dominant activities, such as standing, as it does on the relatively large, but rare strain events of intense (strenuous muscular) activity. From this perspective, the bone loss that accompanies aging or those subject to long-term bed rest may result not only from diminished numbers of high strain magnitude cycles, but also diminished numbers of low strain magnitude cycles caused by the muscle wasting (sarcopenia) that parallels these conditions.⁽⁵⁷⁾

The anatomical substrate for these actions of muscles on bones is not entirely clear. On one hand, tendons determine the translation of muscle force to the bone through the periosteum.⁽⁵⁸⁾ It is believed that muscle growth produces stretching of collagen fibers and periosteum at the muscle/bone interface, which stimulates local periosteal growth.⁽⁵⁹⁾ For example, inactivating mutations of the myostatin gene are associated with hypermuscularity, an increased size of the spinous processes, and expansion of entheses on both the femur and humerus.⁽⁶⁰⁾ On the other hand, specifically in entheses, the gradual transition between tissue types with distinctly different elastic moduli is thought to enhance the ability of tendons to dissipate force evenly during muscle contraction, thus resisting shear stresses at the bone surface.⁽⁶¹⁾ Because blood flow to the limb is proportional to lean (muscle) mass,⁽⁶²⁾ one may expect that haversian perfusion will also benefit from well-developed musculature.

As noted by Pearson and Lieberman,⁽²⁾ most of the available data in both adults and children concern changes in BMD related to exercise and measures of lean mass; a smaller subset of experimental data illuminates the effects of exercise on the cross-sectional geometry of long bones.^(63,64) The study of the relationship between the muscle and bone morphology and especially cortical geometry probably will be even more important than the relation between the muscles and BMD/BMC from the biological and biomechanical perspective, because of the following reasons:

Measures of cross-sectional geometry provide a robust way of assessing the summed effects of modeling on a bone and provide a good indication of the bone's ability to resist mechanical loads.⁽²⁾

Direct stimuli on the periosteum (independent of tendons/entheses) are more "proximal" to the muscles, meaning that shape adaptations to the effects of loading will be reflected there more clearly.

BMD is less useful than measures of cross-sectional geometry, because it does not provide information about the surfaces or compartments in a bone that have grown or been resorbed in response to mechanical loading.

Trabecular architecture, which is equally important to bone geometry for the determination of bone strength,⁽⁶⁵⁾ is not well captured by DXA; therefore, the relevant knowledge is limited. We will thus focus most of the following arguments on cross-sectional bone geometry.

Physical activity in older age

Aging is typically accompanied by declines in physical activity. Some relate it to energy deficiency with aging.⁽⁶⁶⁾ The general view may still be described as "increasing skepticism that exercise can add significant bone mass in adults, and mounting indirect evidence that the consequence of physical activity with regard to the adult skeleton is conservation, not acquisition."⁽⁶⁷⁾

Mechanical loads, greater than those habitually encountered by the skeleton, activate new bone formation on cortical and trabecular surfaces: strain can activate bone cells, which respond with gene activation, increased metabolism, growth factor production, and matrix synthesis.^(28,68) In doing so, these loads increase bone strength and the resistance to fracture.⁽⁶⁸⁾ For example, mechanical stimulation of bone cells may induce elevated levels of IGF1, which promotes bone formation, and stimulate the differentiation of osteocytes from osteoblasts.⁽⁶⁹⁾ There are few studies that have examined whether discrete, localized loads such as those created by muscle contractions on attachment sites have local osteogenic effects. In one study, tensile forces induced the production of IGF1 and IGF1 receptors and proliferation of osteoblast-like cells at the site of load application.⁽⁷⁰⁾ Thus, a leading contemporary theory suggests that both decreasing activity and muscle mass are the main causes of age-related bone loss.⁽⁷¹⁾ An example of a vicious circle has been suggested: less muscle force induces more bone resorption followed by more fractures, then bed rest/inactivity, decreased physical activity, and further decrease of muscular forces.⁽⁷²⁾ Age-related decreases in the range of muscle contractions seem to represent a loss of a key regulatory stimulus.⁽⁷³⁾ Thus, an older inactive individual has neither high magnitude strain signals because of the lack of exercise nor low magnitude strain signals because of muscle fiber loss and limited standing activities.

It is well known that adult bone is less sensitive to exercise-induced changes in peak muscle strain than younger, growing bone.⁽⁷⁴⁾ Thus, in a population-based study of 375 women and 325 men (age, 21–97 yr), Melton et al.⁽⁷⁵⁾ failed to find a close correspondence between physical activity/muscle mass and changes in bone strength with aging. Moreover, interindividual variation in the strength-to-load ratios was substantial,⁽⁷⁵⁾ pointing out that individual intrinsic—probably genetic—factors must exist.

Evidence for genetic determination of bone and muscle mass/strength and genetic pleiotropy

Genetics of bone mass and geometry: Risk factors for osteoporosis (bone mass and structure/geometry) and sarcopenia (muscle mass and strength) are heritable. Genetic factors substantially contribute to variation in bone mass at different sites.⁽⁷⁶⁾ To better understand the genetic origins of hip and vertebral fractures, it is also important to examine heritable determinants of bone geometry. A wealth of studies have documented bone geometry to be under substantial genetic control with 30–70% heritability.^(77–80) Genome-wide linkage analyses of femoral geometry using plain radiographs^(79,81) and DXA-derived hip geometry

TABLE 1. CORRELATIONS BETWEEN AGE-ADJUSTED LEG LEAN MASS AND HIP GEOMETRY IN THE FRAMINGHAM OSTEOPOROSIS STUDY SAMPLE

Geometric trait	ρ_P	ρ_E	ρ_G
NSA	0.213*	-0.075 ± 0.090	0.134 ± 0.110
FNL	0.534*	-0.076 ± 0.092	$0.484 \pm 0.082^*$
NN diameter	0.604*	$-0.298 \pm 0.119^\dagger$	$0.594 \pm 0.069^*$
NN CSA	0.674*	$0.227 \pm 0.088^\dagger$	$0.556 \pm 0.067^*$
NN Z	0.731*	$0.236 \pm 0.084^\dagger$	$0.610 \pm 0.070^*$
Sh diameter	0.599*	-0.087 ± 0.118	$0.751 \pm 0.052^*$
Sh CSA	0.786*	$0.339 \pm 0.086^\dagger$	$0.667 \pm 0.056^*$
Sh Z	0.844*	$0.311 \pm 0.096^\dagger$	$0.806 \pm 0.039^*$

Values with no footnote symbol are nonsignificant ($p > 0.1$).

* $p < 0.0001$.

$^\dagger 0.0001 < p < 0.05$.

ρ_P , phenotypic; ρ_G , genetic; ρ_E , environmental.

traits^(80,82,83) have identified several quantitative trait loci (QTLs) governing the variation in femoral neck geometry. Multiple studies of animals have also supported the above findings of high heritability of bone geometry.^(84,85) For example, several studies using inbred mouse or rat strains have found distinct genetic contributions to vertebral area.^(86–88) Several QTLs in mouse models^(65,89,90) showed a convergence with syntenic loci in humans.

Genetics of muscle mass: Studies have shown that muscle strength and muscle mass are genetically determined.⁽⁹¹⁾ Thus, in young brothers, Huygens et al.⁽²³⁾ found a heritability (h^2) of muscle cross-sectional area (CSA) and mass of ~70–90%. Similarly, h^2 up to 80% was found for DXA-measured lean mass in female twin pairs.⁽⁹²⁾ In the Diabetes Heart Study families, h^2 estimates of whole body lean mass (60%) and appendicular lean mass (66%) measured by DXA were similarly high,⁽⁹³⁾ suggesting that muscle strength/muscle mass are highly transmissible irrespective of measurement technology. Of note, heritability of lean body mass in mice has also been found to be in the same range, ~60%.⁽⁹⁴⁾

Evidence for shared genetics between bone and muscle mass

Given that muscle cells and osteoblasts derive from a common mesenchymal precursor and that muscle and bone are directly connected to each other, it is plausible that there would be potential genes determining both traits. High genetic correlation has been reported between femoral geometric parameters and total body lean mass in U.S. white adults from Nebraska, ranging from 0.28 to 0.69.⁽⁹⁵⁾ Furthermore, the most recent bivariate genome linkage analysis found in that same sample, two chromosomal regions, 5q35 and 10q24, with pleiotropic effects on these phenotypes.⁽⁹⁶⁾ Our preliminary results in the Framingham Osteoporosis Study showed high bivariate genetic correlations between leg lean mass and cross-sectional femoral geometry, ranging from 0.56 to 0.81 (Table 1).

Animal models confirm the above theoretical constructs and empirical observations.⁽⁹⁷⁾ Thus, inactivating mutations of the myostatin (*growth differentiation factor 8* [*GDF8*]) gene induce a hypermuscular phenotype in cattle and mice.

pQCT data showed that myostatin-knockout mice have significantly greater cortical BMC at L_5 than normal mice,⁽⁸⁸⁾ as well as an increased size of the spinous processes and expansion of entheses on both the femur and humerus.⁽⁶⁰⁾ In contrast, mice with the “mini-muscle phenotype” (homozygous for a mendelian recessive allele that halves hind-limb muscle mass⁽⁹⁸⁾) exhibited significantly longer and thinner femora and tibia/fibulae, without a difference in bone mass.⁽³⁸⁾ Finally, QTLs have been identified for traits related to both bony carcass and meat quality in Scottish sheep⁽⁹⁹⁾; these traits were found to correlate with genes also in beef cattle.⁽¹⁰⁰⁾

Implications for the genetic studies of osteoporosis: a need to consider pleiotropy

As substantiated above, there is abundant evidence suggesting that osteoporosis is largely determined by genetics.^(101,102) As reviewed by Ioannidis et al.,⁽¹⁰³⁾ by 2004, >60 QTLs had been identified from 20 human genome scans of BMD and/or fracture, covering all chromosomes except Y, but not many have been replicated in studies of independent groups or in a meta-analysis. The relative lack of success in identifying genes for osteoporosis despite a plethora of recent studies may have resulted, in part, from an inadequate phenotype definition. Progress in the genetics of bone fragility is slow because the phenotype is poorly defined.⁽¹⁰⁴⁾ The risk of osteoporotic fracture can be viewed as a function of loading conditions and the ability of human bone to withstand the load. Risk of fracture is not entirely determined by intrinsic properties of bone, as other extrinsic factors are known to contribute (sarcopenia, fall-related factors, including impaired cognition, and sensory input); there is thus an increasing realization that osteoporosis does not explain all low trauma fractures.⁽¹⁰⁵⁾ It is also important to remember that the genetic contribution to a risk factor may differ from the genetic contribution to the ultimate disease phenotype. For example, DXA-derived aBMD, which has been commonly used as an endophenotype for fractures caused by osteoporosis, was found to share only modest genetic variance with osteoporotic fractures.^(106–109) Therefore, neither aBMD alone or bone structure are perfect surrogates of the skeleton's ability to withstand the forces that produce fracture. Also, the most important clinical outcome of any anti-osteoporosis intervention is a reduction in the risk of fractures. If this is achieved by increasing bone strength, this will be important; if this is done by decreasing fall rates, this will be even more beneficial for other outcomes of falls.

Phenotype definition is therefore an important consideration for any genetic epidemiological study. In addition, there is a need to take into account the multivariate etiology of osteoporosis. We postulate that to achieve success in the study of the genetics of osteoporosis (or genetics of sarcopenia), one needs to undertake a pleiotropic approach. There are several incentives for studying the contribution of genetics to both muscles and bones jointly, and there is both theoretical and empiric potential for success of this approach for several reasons. First, the “postgenomic” era provides the opportunity to use a technically advanced

hypothesis-free selection method to identify the major genetic variants. Second, the computational tools, exhaustive databases, and advanced database querying methods support the conduct of multivariate and multidisciplinary analyses. Comprehensive phenotyping will allow for broader exploration of contributing genes and should be particularly valuable for newly proposed analytic approaches, such as reverse phenotyping.^(110,111) This approach, titled “applied phenomics,” would redefine a phenotype of a syndrome or condition based entirely on genetic covariance between the traits (which may not be obvious at the phenotypic level) and to embark on the theoretically increased power of performing joint analyses of genetically correlated traits.^(112–114)

Current approaches to determining genes that influence complex traits

There have been two different strategies to explore the genetic basis of complex traits (other than mendelian syndromes), each with its own advantages and pitfalls. The first strategy is genome-wide, sometimes referred to as forward genetics. Because of recent technological advances in genome sequencing, the increased knowledge gained from the HapMap project,⁽¹¹⁵⁾ and the refinement of bioinformatics, this strategy has been significantly facilitated with the use of microsatellite markers and at present, genome-wide SNP chips. A newly available approach to reach that goal, genome-wide association (GWA), analyzes SNPs across the whole genome^(116,117) to provide robust de novo identification of chromosomal regions/SNPs that are linked and associated with the traits of interest. A second strategy (reverse genetics) is to pursue candidate genes through association studies. The candidate gene approach needs a strong prior hypothesis and has proven viable when there is a strong biological basis for inclusion of a gene as a plausible candidate. It seems that exploring several candidate genes belonging to the signaling pathways/gene regulation network with biological relevance to both bones and muscles will be a more promising strategy.

The majority of candidate genes traditionally come from basic studies in cell cultures and animal models. However, with the advent of genome-wide scans,⁽⁹⁶⁾ expression experiments,⁽¹¹⁸⁾ and bioinformatic tools,⁽¹¹⁹⁾ new candidate genes may emerge for further pursuit.

Candidate genetic mechanisms for bone geometry and muscle mass

Numerous candidate genes for bone geometry have been proposed as contributors to osteoporosis risk based on bone biology.^(120–122) Volkman et al.⁽¹²³⁾ proposed possible pathways through which genes may influence bone structure and geometry: (1) direct influence on bone size and shape (i.e., directed activity of osteoblasts and osteoclasts); (2) indirect effect on factors such as body weight, muscle strength, and activity level, which alter mechanical load on bones⁽¹²⁴⁾; and (3) effect on responsiveness of bone to applied mechanical loading (i.e., set points in a “mechanostat”-like mechanism⁽²⁸⁾). We will focus on the latter two scenarios, which involve the key genetic pathways and

single genes that apparently have a biologically plausible pleiotropic effect on both bone geometry and muscles. There are several parallel and partly complementary genetic systems involving intertwined hormonal functions for a muscle–bone interaction because these need to be both pleiotropic and multigenic for safety reasons.^(29,33) In Table 2, we provide several pathways and single genes, known as candidates for osteoporosis, for which there are indications of pleiotropic actions also on lean mass/muscles. Of note, this is not a comprehensive review of the literature or a formal search of the relevant databases; these genes were identified by a PubMed search using keywords such as “osteoporosis,” “sarcopenia,” “gene,” or “polymorphism,” etc. Only those genes for which more than a single reference was available are listed in Table 2.

Sex hormones: Sex hormones are obvious candidates for regulation of both bones and muscles because the sexual dimorphism in bone geometry/mass and muscle strength is pronounced. There are two primary groups of sex hormones: androgens and estrogens. The skeletal actions of androgens may result from direct activation of the androgen receptor (AR) or may alternatively depend on stimulation of the estrogen receptors (ESRs) after aromatization of androgens into estrogens that occurs in peripheral tissues (including bone); the enzyme, aromatase, catalyzes this conversion. These receptors are essential for normal skeletal growth and bone mineral acquisition,⁽¹²⁵⁾ as well as being important regulators of recovery from disuse atrophy.⁽¹²⁶⁾ Sex hormones or related factors could affect the threshold of the feedback system that controls bone remodeling to adapt bone structure to the strains derived from customary mechanical usage in each region of the skeleton (through a bone “mechanostat”).^(29,43,127)

Genetic polymorphisms: AR polymorphisms occur commonly in the population and are the best-characterized genetic determinants of androgen responsiveness. The length of the trinucleotide repeat (CAG_n) in exon 1 is inversely associated with transactivation activity because of reduced binding of AR co-activators and can modulate physiological androgen effects, such as prostate size, sperm concentration, concentrations of lipids, insulin, and leptin, and BMD.⁽¹²⁸⁾ Polymorphisms in two estrogen receptors, *ESR1* and *ESR2*,^(129–131) as well as in the aromatase gene *CYP19*,^(125,132) have been extensively studied. Studies suggested that some variants in *ESR1* cause less sensitivity to estrogen, because of differential expression of the receptor.⁽¹³³⁾ The retinoblastoma-interacting zinc finger protein (*RIZ1*) has been shown to function as a specific estrogen nuclear hormone receptor co-activator and to strongly enhance the function of the ESR1 both in vivo and in vitro; a deletion polymorphism in the *RIZ1* gene exhibits decreased response to estrogen in vitro.^(134,135)

Another candidate that has been studied with regard to bone and muscle effects is catechol-*O*-methyltransferase (*COMT*), an estrogen-degrading enzyme, also involved in the degradation of catecholamines. The *COMT* val158met polymorphism was associated with peak BMD in young men,⁽¹³⁶⁾ and most recently, an interaction of COMT with physical activity was found in the same young men.⁽¹³⁷⁾ Also, *CYP17A1*, the cytochrome P450 family 17 subfamily

TABLE 2. CANDIDATE GENES AND PATHWAYS* FOR PLEIOTROPIC ACTION ON BONE AND MUSCLE

<i>Gene (abbrev.)</i>	<i>Gene title</i>	<i>Action</i>	<i>Reference</i>
Sex hormones			
<i>AR</i>	Androgen receptor	Decreased activity (e.g., with administration of an <i>AR</i> antagonist or in <i>AR</i> -gene knockout mice) results in a loss of bone mass	(173)
		<i>CAGn</i> repeats associated with fat-free mass in men	(174)
<i>ESR</i>	Estrogen receptor	In a meta-analysis, <i>XbaI</i> polymorphism associated with BMD and fracture risk in women	(175)
		<i>Esr1</i> knockout mice unable to respond to physical exercise with a periosteal bone expansion as seen in wildtype mice	(176)
Growth hormone/insulin-like growth factor pathway			
<i>IGF1</i>	Insulin-like growth factor I	CA-repeat promoter polymorphism has effects on femoral bone geometric parameters	(121)
		Associated with increased bone strength and muscle volume and strength	(177)
		Alternative splicing involved in the mechanotransduction of bone cells	(144,145)
Myostatin pathway			
<i>GDF8</i>	Myostatin	Myostatin-null mice had significantly greater cortical bone mineral content and larger entheses than normal mice	(60,88)
		In humans GDF8 mRNA levels were reduced in response to heavy-resistance strength training	(148)
Vitamin D signaling pathway			
<i>VDR</i>	Vitamin D receptor	In meta-analysis, <i>Cdx-2</i> polymorphism associated with risk for vertebral fractures in women	(152)
		<i>BsmI</i> polymorphism associated with decreased vertebral area and femoral narrow neck width	(154)
		<i>BsmI</i> polymorphism associated with muscle strength	(155,156)
		<i>FokI</i> polymorphism associated with fat-free mass and sarcopenia in older men	(178)
Wnt/LRP5 pathway			
<i>LRP5</i>	Low-density lipoprotein receptor-related protein 5	Mice with nonfunctional Lrp5 receptors had reduced bone formation in response to mechanical loading, compared with controls	(172)
		In humans, genetic variation modulated the relationship between physical activity and BMD	(124)
Inflammatory cytokines			
<i>IL6</i>	Interleukin 6	-174 GC polymorphism associated with increased risk of wrist fracture in postmenopausal women	(161)
		-174 GC associated with hip BMD in postmenopausal women	(162)
		-174 GC associated with fat-free mass in men but not women	(163)
Other pathways(†)			
<i>GCR</i>	Glucocorticoid receptor	Contributes both to bone and lean mass in older persons, muscle strength in younger males	(179,180)
		Associated with lower trochanteric BMD in elderly women	(181)
<i>LEP</i>	Leptin	Plasma leptin concentration was correlated with BMD in both sexes	(182)
		Plasma leptin concentration was correlated with hand bone size and geometry	(183)
		<i>LEP</i> gene regulates carcass and meat quality traits of beef cattle	(100)
<i>PTN</i>	Pleiotrophin	Overexpression effects on mouse long bone development, fracture healing and bone repair	(184)
		Potential mediator of mechanotransduction signaling pathways in regulating periosteal bone formation and resorption in mouse	(185)
<i>RETN</i>	Resistin	Serum levels showed a significant negative correlation with lumbar spine BMD in middle-aged men	(186)
		Polymorphisms associated with muscle and bone phenotypes in men and women	(187)
<i>SRY-17</i>	Transcription factor SRY (sex determining region Y)-box 17	Involved in the endochondral bone growth	(188)
<i>VEGF</i>	Vascular endothelial growth factor	Downregulated in older men (as a part of the “sarcopenia signature”)	(118)
		Couples hypertrophic cartilage remodeling, ossification, and angiogenesis during endochondral bone formation	(189)
		Potential role for vasculogenesis in adult muscle	(190)

* Pathways may overlap (the same gene may belong to more than one pathway).

† There are limited references for these genes.

A gene, has been considered as a strong candidate gene for osteoporosis, because of its role in coding for a key enzyme in the steroidogenic pathway that produces progestins, mineralocorticoids, glucocorticoids, estrogens, and androgens.⁽⁹⁶⁾

IGF1: Systemic control hormones, GH and/or its related factors, are active during development and repair to generate morphological change in bones in a fully coordinated manner with the skeletal muscle.⁽³⁴⁾ They are also active during adult ontogenesis. GH activates *IGF1* gene transcription in vivo; GH treatment upregulates the level of *IGF1* expression.⁽¹³⁸⁾ IGF1 in turn plays a prominent role in the pathophysiology of osteoporotic fracture. Several reports^(139,140) have documented a decrement in IGF1 plasma levels with age in humans. Mechanical stimulation on bone cells may induce elevated levels of IGF1, which in turn prompts the differentiation of osteocytes from osteoblasts and thus promotes bone formation, for example, in rat calvaria.⁽⁷⁰⁾ The relevance of IGF1 for cortical bone metabolism is also well established in the mouse.^(141,142)

A CA-repeat promoter polymorphism in the *IGF1* gene was found to be associated with circulating IGF1 levels.⁽¹⁴³⁾ Also, Goldspink et al.⁽¹⁴⁴⁾ cloned two different IGF1 isoforms that are expressed by skeletal muscle, and both are derived from the *IGF1* gene by alternative splicing (reading frameshift change). One of these isoforms, called “mechanogrowth factor” (MGF), is expressed in response to physical activity and is apparently involved in the mechanotransduction of bone cells.⁽¹⁴⁵⁾ The other is similar to the systemic or liver type and is important as the provider of mature IGF1; its expression is also upregulated by exercise. It seems that these two forms of IGF1 have somewhat different actions and that they both are important regulators of muscle growth and therefore contribute to bone strength. Table 2 presents data on pleiotropic action of this and other polymorphisms. IGF1 induces muscle hypertrophy by activating the IGF1 receptor (IGF1R), which activates multiple signaling pathways, including the PI3K and MAPK pathways. In addition to *IGF1*, the *IGF-2* genotype also was associated with total body fat-free mass in humans⁽¹⁴⁶⁾ and muscle growth in pigs.⁽¹⁴⁷⁾

Myostatin: Another interesting candidate is myostatin (*GDF8* gene), a member of the TGF- β superfamily and a negative regulator of skeletal muscle mass. Inactivating mutations of the *GDF8* gene induce a hypermuscular phenotype in cattle and mice.⁽⁶⁰⁾ Strength training suppresses myostatin expression, showing that downregulation of myostatin signaling is one mechanism by which resistance exercise increases muscle mass.⁽¹⁴⁸⁾ Findings in *mdx* mice (a model for Duchene muscular dystrophy) show that myostatin blockade can simulate the effects of strength training. Myostatin binds to an activin type II receptor and initiates a signaling cascade that involves the activation of Smad proteins (which is another possible pathway for the *GDF8* involvement in the skeleton). Most recently, it was also indicated that myostatin may act upstream of Wnt pathway components; specifically, it repressed expression of Wnt4 in the skeletal muscle.⁽¹⁴⁹⁾ Table 2 presents data on pleiotropic action of the *GDF8* on bone.

Vitamin D receptor

The vitamin D receptor (*VDR*) gene has long been targeted as one of the genetic determinants influencing bone status because it regulates bone homeostasis through the vitamin D endocrine system. There are a few studies that have examined the associations between *VDR* and muscle strength; Bischoff et al.^(150,151) showed expression of the *VDR* in human skeletal muscle tissue, which decreases with age. Also, short-term vitamin D supplementation does reduce body sway, in addition to an improvement in lower body strength, which is reduced in vitamin D deficiency. This reduction in the number of falls and fractures would indicate an alternative mechanism other than bone strength/BMD, and one option would be a relationship between *VDR* polymorphisms and muscle strength.

A number of SNPs within this well-known and widely studied gene for osteoporosis have been studied, including the *BsmI*, *ApaI*, and *TaqI* polymorphisms located in the 3' end of the gene⁽¹⁵²⁾ or the Cdx-2 transcription factor binding site, which is located within the promoter region.⁽¹⁵³⁾ Table 2 provides evidence for associations of *VDR* polymorphisms with bone geometry,⁽¹⁵⁴⁾ fractures,⁽¹⁵²⁾ and muscle mass or strength.^(155,156)

Low-density lipoprotein receptor-related protein 5

Polymorphisms in the low-density lipoprotein receptor-related protein 5 (*LRP5*), a Wnt co-receptor, have been associated with BMD in men and women.^(122,157) In mice, Akhter et al.⁽¹⁵⁸⁾ found that the *Lrp5* G171V mutation results in a skeleton that has greater structural (femoral shaft, femoral neck, tibiae, vertebral body) and apparent material (vertebral body) strength. They hypothesized that the denser and stiffer bones in G171V mice may represent greater sensitivity to normal mechanical stimuli, resulting in an overadaptation of the skeleton to weight-related forces. More recent studies suggest that, whereas knockout mice lacking the protein can sense mechanical load correctly, they are unable to respond properly to it by forming new periosteal bone.⁽¹⁵⁹⁾ In our study,⁽¹²⁴⁾ genetic variation in polymorphisms of the *LRP5* gene was found to modulate the relationship between physical activity and BMD in men, thus suggesting that *LRP5* may play a role in the adaptation of bone to mechanical load in humans as well. Furthermore, Wnt signaling has been implicated in overload-induced skeletal muscle hypertrophy in mice.⁽¹⁶⁰⁾ All things considered, this may suggest that Wnt signaling, and specifically, *LRP5* may be involved in muscle/bone cross-talk. As mentioned above, myostatin may act on both bone and muscle tissue upstream of Wnt signaling.⁽¹⁴⁹⁾

Other genes with possible pleiotropic actions may exist. Actions of IL-6 in bone and muscle loss also become apparent.⁽²⁶⁾ IL-6 is a cytokine that has been shown to be produced at high levels by human skeletal muscle after endurance-type exercise, having a role in the mobilization of substrates to support metabolism during exercise. The best studied with osteoporosis-related traits is a SNP G/C at -174 bp, which was shown to be associated with increased risk of wrist fractures⁽¹⁶¹⁾ and lower hip BMD in postmenopausal women⁽¹⁶²⁾ and fat-free mass in men but not

women.⁽¹⁶³⁾ In vitro studies showed that IL-6 inhibits the secretion of IGF1; thus, the negative effect of IL-6 on muscle function might be mediated through IGF1.⁽¹⁶⁴⁾

In summary, Table 2 provides indications that genes from multiple pathways, including inflammatory, GH, and steroid metabolism, are candidates for pleiotropic regulation of bone and muscle. Other possible candidates such as leptin, transcription factor SRY-box 17, pleiotrophin, vascular endothelial growth factor, and glucocorticoid receptor, are also shown in Table 2; for these genes, less is known about their pleiotropic action on bone and muscle. This list needs to be refined by bioinformatic search of genetic association and expression databases and confirmed through functional studies.

Additional considerations: gene-by-gene interactions on bone strength and muscle mass

Bone and muscle anabolism can be induced by multiple pathways. There are several parallel and partly complementary physiological feedback systems involving intertwined hormonal functions for the muscle–bone interaction.^(29,33) Any list of the candidate genes may be either incomplete or redundant. We may thus expect that, in addition to pleiotropic effects of the genes, there are gene-by-gene interactions among these pathways; thus, epistatic pleiotropy may be involved.

Indeed, epistatic interactions between *ESR1*, *ESR2*, and nuclear receptor interacting protein 1 (*NRIP1*) genes were associated with osteoporosis in postmenopausal Spanish women.⁽¹⁶⁵⁾ Also, sex steroids interact with the GH/IGF1 axis during puberty,⁽¹⁶⁶⁾ which may be caused by utilization of the same signal transduction pathways, including MAPKs and PI3K. There is an indication that testosterone increases concentrations of IGF1 and reduces concentrations of IGF1 inhibitory proteins.⁽¹⁶⁷⁾ Alternatively, systemic IGF1 and AR-signaling may stimulate periosteal growth independently.⁽¹⁶⁸⁾ Also, Rivadeneira et al.⁽¹⁶⁹⁾ found that variants of *ESR2* in interaction with *ESR1* and *IGF1* influenced femoral neck cortical thickness and section modulus in postmenopausal women.

These gene interactions add a degree of complexity to the relationships among the genes in the anabolic pathways chosen above as examples. This also strongly suggests a need to explore the interactions, at least between the genes belonging to the same pathway. The question of interactions between multiple genes is thus an intriguing one that requires more elaborate study designs.

SUMMARY AND RECOMMENDATIONS.

In conclusion, there are many more questions than answers. It is not fully understood how bone senses and responds to muscle actions, whether it loses its mechanosensitivity with aging, or which cells are responsible for this ability. Currently not much is known of the role endogenous estrogens and administration of estrogen may play in the management of sarcopenia; estrogen replacement therapy (ERT) did not prevent loss of muscle composition and strength with aging.^(170,171) Similarly, the role of aro-

matization of testosterone into estrogen in mediating the testosterone effects on body composition/muscle mass is poorly understood.

A joint approach focusing on common genetic determination of both skeletal geometry and muscular mass will identify new signaling pathways, which in turn will pinpoint novel biological mechanisms. The study of model organisms and whole genome expression and candidate gene association that deals with two outcomes, muscular and osseous, should prove helpful in this task.

It is important to consider dose-response depending on a threshold of exercise necessary to maintain the muscle and bone. Future work should attempt to document whether such a threshold exists and, if it does, to determine the loads necessary to induce osteogenesis.⁽⁵³⁾ There is a need to create a drug/intervention able to reverse the aging of bone, to make bones more susceptible to beneficial effects of exercise, muscle load, and stimulation through vibration, comparable of that of young subjects. This type of therapy could be used to reverse sarcopenia in the elderly, to prevent muscle loss that occurs in astronauts,⁽¹⁷²⁾ and to combat falls and fractures. The progress in these studies may translate into new approaches to the prevention of hip and vertebral fractures as well as muscle wasting; moreover, it may ultimately help understand broader concepts, such as frailty.

We postulate that to achieve success in the study of osteoporosis (as well as sarcopenia), there is a need to take into account the multivariate nature of the complex diseases and to capitalize on benefits of the pleiotropic approach. Identifying significant genetic variants underlying both bones and muscles, measured with state-of-art technology and replicated in large human cohorts and animal experiments, will provide valuable insight into important potential targets for risk stratification, as well as pharmacogenetic applications. Knowledge of the genetic machinery underlying an interaction between bones and muscles may ultimately identify targets for specific interventions aimed at increasing bone strength, muscle strength, or both.

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APPENDIX: GLOSSARY

Allometric: relates to the proportions of various parts of an organism as a consequence of growth and development, for example, the change of body parts in direct proportion to body size

Alternative splicing: a regulatory mechanism by which different forms of mature mRNA are generated from the same gene to produce more than one related protein (isoform), by variations in the incorporation of the exons, or coding regions, into mRNA

Endophenotype: a measurable trait that is more proximate to the pathophysiologic effects of the gene. For example, in the case of osteoporosis, endophenotypes will be bone turnover markers, whereas BMD, muscle mass, etc., are “intermediate” phenotypes

Epigenetic: any factor that indirectly influences the expression of the genome without directly affecting its DNA, for example, DNA methylation and chromatin remodeling

Epistasis: two or more genes interacting with one another in a multiplicative fashion

Gene expression: the process of converting the genetic information encoded in the DNA into the final gene product

Heritability(h^2): In the narrow sense, h^2 is defined as the proportion of the total phenotypic variance in a trait that is caused by the additive effects of genes, when environmental factors are excluded. In the broad sense, heritability is proportion of the total phenotypic variance of a trait that is caused by all genetic effects, including additive and dominance effects.

Knockout mice: mice in which a gene has been deleted or inactivated in both the somatic and the germ cells so that the animals produce no functional gene product

Phenotype: observed physical, biochemical, structural, or physiological manifestation of a genotype

Pleiotropy: one gene leading to many different phenotypic expressions

Polymorphism: any locus at which at least two alleles are available, such that at least 1% of a population carries the rarer genotype

Quantitative trait: a phenotype that varies in a quantitative manner, which can be caused by combinations of genetic and environmental factors, as well as a measurement error. Quantitative traits are often considered to be polygenic traits.

Reverse phenotyping: a computational process by which genetic data used to drive new phenotype definitions (in silico)

Strain: deformation of a material divided by a corresponding undeformed dimension (e.g., 1 microstrain = 10^{-6} m/m or in/in)